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Current Opinion in Chemical Biology

Antivirulence as a new antibacterial approach for chemotherapy

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Bacterial resistance to antibiotics is an issue that has led to the search for new antibacterial approaches. Drugs targeting virulence is an alternative approach to treat infections due to resistant bacteria. There is extensive literature and knowledge in the field of bacterial pathogenesis and genomic determinant of virulence. As therapeutic targets, virulence factors have been primarily addressed in the vaccine field to prevent infection by specific pathogens. Recently novel strategies to identify virulence inhibitors have been numerous and several new compounds were recently reported. This review emphasizes the new virulence inhibitors that have shown a biological activity and have made a proof of concept that disarming bacteria lead to the inhibition of bacterial infection in experimental models *in vivo*. Moreover, some of these new antivirulence compounds are able to inhibit the virulence of different related pathogenic species, indicating that it is possible to target common virulence mechanisms. The progress reported recently with proof of concept for antivirulence molecules at the preclinical stages should allow the antivirulence concept to become a reality as a new antibacterial approach.

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Current Opinion in Chemical Biology 2008, **12**:400–408

This review comes from a themed issue on
Next-generation therapeutics
Edited by Floyd Romesberg and Anna Mapp

Available online 17th July 2008

1367-5931/$ – see front matter

DOI 10.1016/j.cbpa.2008.06.022

Introduction

The increasing antibiotic resistance of most clinically relevant bacteria creates an urgent need for new antibacterial classes that are not affected by resistance mechanisms already present in the bacterial population [1,2].

Very few new antibiotics with new mechanisms of action have been found in the recent past [3,4,5,6•,7]. The mechanisms of resistance spreading in pathogenic bacterial populations call for the inhibition of new bacterial targets [4,8,9••,10]. The inhibition of virulence targets could bring new antibacterial molecules with radically new mechanisms of action and represent an innovative therapeutic concept [11,12]. Virulence is defined as the relative capacity of a microbe to cause damage in a host.

Although this definition is simple, it does encompass a wide variety of bacterial functions such as the direct effectors of pathogenicity (e.g. toxins), and many of the functions that are not essential for basic metabolism (*in vitro* essential genes) that contribute to the establishment of an infection in the host and are validated by deletion mutants (essential *in vivo*).

Antivirulence compounds could have advantages over classic antibiotics in two key ways. Firstly, the targets or pathways that are inhibited: Rather than target genes that are essential for basic metabolism *in vitro*, the targets are essential functions for host/pathogen interactions, which allow bacterial multiplication in the host. Therefore, the selective pressure for viability of mutants potentially carrying a resistance genotype should be limited to the host tissues where and when the targeted virulence factor is indispensable for bacterial survival. Secondly, the specificity of the effect (i.e. activity against bacteria that are causing pathogenesis via dissemination in the host) should preserve the bacteria constitutive of the normal flora.

With genomics and the diverse *in vivo* gene expression technologies available [13,14,15] our knowledge of bacterial infection physiopathology is improving [16,17]. It has permitted the identification of many new genes and metabolic pathways essential for a bacterium to cause infection of the host. Of particular interest are the bacterial targets that are conserved in several pathogenic species. To establish an infection and produce a disease, pathogenic bacteria have developed different virulence mechanisms in order to colonize, disseminate, adapt to the various environments in the host, subvert host functions, invade the host tissues, and overcome host defenses [18]. These different stages of pathogenesis represent a variety of pharmacological targets for inhibitors.

Inhibition of adhesion and colonization

The literature has shown that the prevention of bacterial adhesion to host cells could be achieved by inhibition of pili formation. Peptide mimetics that were able to bind the PapD protein, a conserved chaperone for pilus assembly in uropathogenic *E. coli* were designed and these inhibitors induce a decrease piliation of the bacteria [19]. The bicylic-2 pyridone designed by RDD are inhibitors of PapD; they have large spectrum potential and no effect on bacterial growth. They are able *in vitro* to decrease the relative abundance of pili per cell, biofilm formation, and the adherence of UPEC to bladder cells [20].

Virstatin was found by HTS, it is an inhibitor of ToxT a regulator of Cholera toxin expression and of pilus assembly. In an experimental model of infection by *V. cholerae*, Virstatin was able to inhibit virulence factors expression and intestinal colonization [21].

The inhibition of type III secretion systems (T3SS) may block the colonization step since this system is used by the Gram-negative pathogens to inject effectors directly into the host cell. Many of the translocated effector proteins interfere with intrinsic eukaryotic host cell functions to enable internalization of the bacteria in the eukaryotic cell. T3SSs play essential roles in the virulence of a large spectrum of Gram-negative pathogens, including *Chlamydia*, *Escherichia coli*, *Pseudomonas*, *Salmonella*, *Shigella*, and *Yersinia* [22]. Specific inhibitors of conserved effectors of T3SS have been reported. Benzimidazole derivatives (developed by Parateck Inc.) are inhibitors of a transcription factor important for the T3SS regulation of virulence in *Pseudomonas aeruginosa*. They have no direct antibacterial activity *in vitro*, but in a mice pneumonia model of infection, treatments with these compounds increase the survival rate [23].

The salicylidene acylhydrazides (developed by Innate Pharmaceuticals) inhibit *S. typhimurium* T3SS activity. They can also inhibit the *Y. pseudotuberculosis* or *C. trachomatis* T3SS [24]. These new compounds prevent secretion of T3SS effectors, invasion of cultured epithelial cells, and enteritis *in vivo* [25,26].

A benzylidene benzohydrazide able to block the secretion of *Yersinia* Yop effectors by interfering with the activity of the T3SS apparatus was also shown to inhibit *C. trachomatis* intracellular development [27].

Inhibition of the secretion of cell surface proteins

Sortases are required for the pathogenesis of many different bacterial infections and owing to the high degree of conservation in Gram-positive bacteria has been considered a good pharmacological antivirulence target [28,29].

Inhibitors of sortase such as peptidomimetic molecules have been identified by HTS assays [30]. Diarylacrylonitriles inhibitors of Sortase A also exhibited potent inhibitory activity against *S. aureus* cell adhesion to fibronectin [31]. With flavonol inhibitors of *S. aureus* sortase an activity in the bacteria was demonstrated using the clumping assay to fibrinogen [32]. Recently, Aryl-amino ethyl ketones (AAEKs) were shown to inhibit the sortase enzyme of two different bacterial species *S. aureus* and *B. antracis* [33]. This result based on structural design has opened the way for inhibitors with a larger spectrum of activity.

Siderophores are essential for the virulence of pathogenic bacteria. Iron is a limiting nutrient in the blood, and bacteria have developed several siderophore mechanisms for iron uptake. A salicylsulfamoyl adenosine was found to inhibit an enzyme involved in siderophore biosynthesis in *M. tuberculosis* and *Y. pestis*. The inhibitor was shown to inhibit the growth of both bacterial species in iron limiting conditions [34••].

Inhibition of regulatory bacterial function

To adapt to the environment, bacteria use a large array of regulatory pathways and some of them are potential targets for new inhibitors.

Quorum sensing (QS) is a complex regulatory network that governs the expression of a series of bacterial virulence factors in response to cell density or environment changes. In particular, QS influences the ability of bacteria to form complex surface-associated structures such as biofilm and the ability of bacteria to resist clearing by the innate immune system [35]. The signals for QS are molecules called autoinducer (AI), and the promising QS inhibitors (QSI) that block the AI signaling have been reported [36]. In Gram-negative bacteria, QS is mediated by homoserine lactone derivatives. Synthetic derivatives of natural furanones interfere with the QS signals; they decrease QS regulated gene expression in *P. aeruginosa* in mice [37]. Furanone derivatives are able to suppress QS in the lung of mice, to accelerate the bacterial clearance in the lung and to improve survival in mice infected by *P. aeruginosa* [38]. Other QSI such as the halogenated anthranilic acid analogs inhibit a biosynthesis step of QS, and disrupt QS dependent gene expression. These compounds restricted *P. aeruginosa* systemic dissemination and mortality in mice, without perturbing bacterial viability [39]. Treatment, with the QS inhibitor furanone C-30, of mice harboring implants colonized with *P. aeruginosa* accelerates the clearing of the implants [40]. Natural products such as patulin and penicillic acid were shown to be biologically active QSI that increased the sensitivity of bacterial biofilm to trobamycin. In a mouse pulmonary infection model, *P. aeruginosa* was more rapidly cleared from the mice that were treated with patulin compared with the placebo group [41].

In Gram-positive bacteria the QS signal is made of autoinducer peptides (AIPs) that control virulence factors expression and interaction with the immune system [42,43••]. *S. aureus* (like *P. aeruginosa*) modulates virulence factor expression through at least two QS systems that regulate one another (AgR and RAP) [44••]. Inhibition of the AgR regulation was obtained by AIP analogs that antagonize the binding of the AIP to its receptor the Agr C histidine kinase and block the activation of the Agr response and then inhibit the expression of the regulated virulence factors [45]. The QS inhibitor RNAIII-inhibiting peptide (RIP) has already been assessed in multiple

animal models and has been found to have strong activity in preventing staphylococcal infections [46].

Antibodies that have been derived against the AI in *S. aureus* are able to decrease the expression of the proteins regulated by the QS effector AgR, without affecting *in vitro* viability; furthermore, the treatment with the antibodies decreases the skin injury in mice [47••]. These results represent an important demonstration of the feasibility of QS inhibition as a new antibacterial approach.

However, interference with QS in *S. epidermitis* has been reported to enhance biofilm formation [43]. Clinical isolates of *S. aureus* variant with loss of Agr function were found in biofilm, and the correlation between *S. aureus* strains with decreased sensitivity to vancomycin and AgR phenotype has been reported [48,49]. *In vivo* QS activation appears necessary in high density actively growing cells, but for quiescent cells and biofilm formation, QS inactivation appears to be a prerequisite [35,50,51]. These studies indicate that the temporal shift in the expression of QS during the infectious process might represent a challenge to use QSI in the host.

Inhibition of the bacterial cell wall resistance to the innate immunity

The resistance of bacteria to the innate immunity components is mediated by several virulence mechanisms and allows dissemination of the infection in the host.

The components of the innate immunity are either circulating molecules or cells. The molecules are the complement factors, and the antibacterial peptides such as defensins, which could have bactericidal effects by direct interactions with the bacterium cell wall in dividing bacteria [52,53•]. The circulating phagocytes and the polymorphonuclear leukocytes (PMNs) are able to kill invading bacteria after activation by releasing lytic peptides or reactive oxygen species (ROS).

Capsular polysaccharides are protecting bacteria from immune recognition and killing by cell-mediated immunity. Other genes essential to protect the bacteria from the destruction of the membrane integrity by the immune system have been identified and now investigated as virulence targets in Gram-positive and Gram-negative species.

In Gram-negative bacteria the outer membrane serves as the first barrier encountered by peptides and effectors of innate immunity. Lipopolysaccharide (LPS) is the major component of the outer membrane of Gram-negative bacteria. LPS is a major determinant of pathogenicity in Gram-negative bacteria and has been shown to be necessary for resistance to cationic peptides and complement [56].

LPS molecules are negatively charged and consist of the lipid A and core oligosaccharides to which are attached the polysaccharides forming the O Antigens. LPS biosynthesis is a target for antibiotics, and new inhibitors of biosynthetic enzymes have been reported [54,55]

The resistance to cationic peptide is linked to the modification of lipid A, in particular by the addition of aminoarabinose into lipid A to decrease the surface negative charges. The modifying enzyme PmRk/Arnt could be inhibited with small molecules [57•].

The conserved core LPS is necessary for virulence [58,59] and is composed of heptose (Figure 1). Inhibition of heptose biosynthesis appears as a good bacterial target for inhibitors owing to the high degree of conservation in this pathway in Gram-negative bacteria, and inhibitors were found in HTS assays [60]. In Mutabilis we have validated RfaE (a core LPS biosynthesis enzyme) as a good target for inhibition using mutants in pathogenic *E. coli*. The mutants were highly sensitive to the complement factors present in serum, fully avirulent *in vivo* but were still able to colonize the intestine of mice. Promising potent inhibitors of RfaE enzyme were found using HTS and will be developed to render the bacteria sensitive to complement [61].

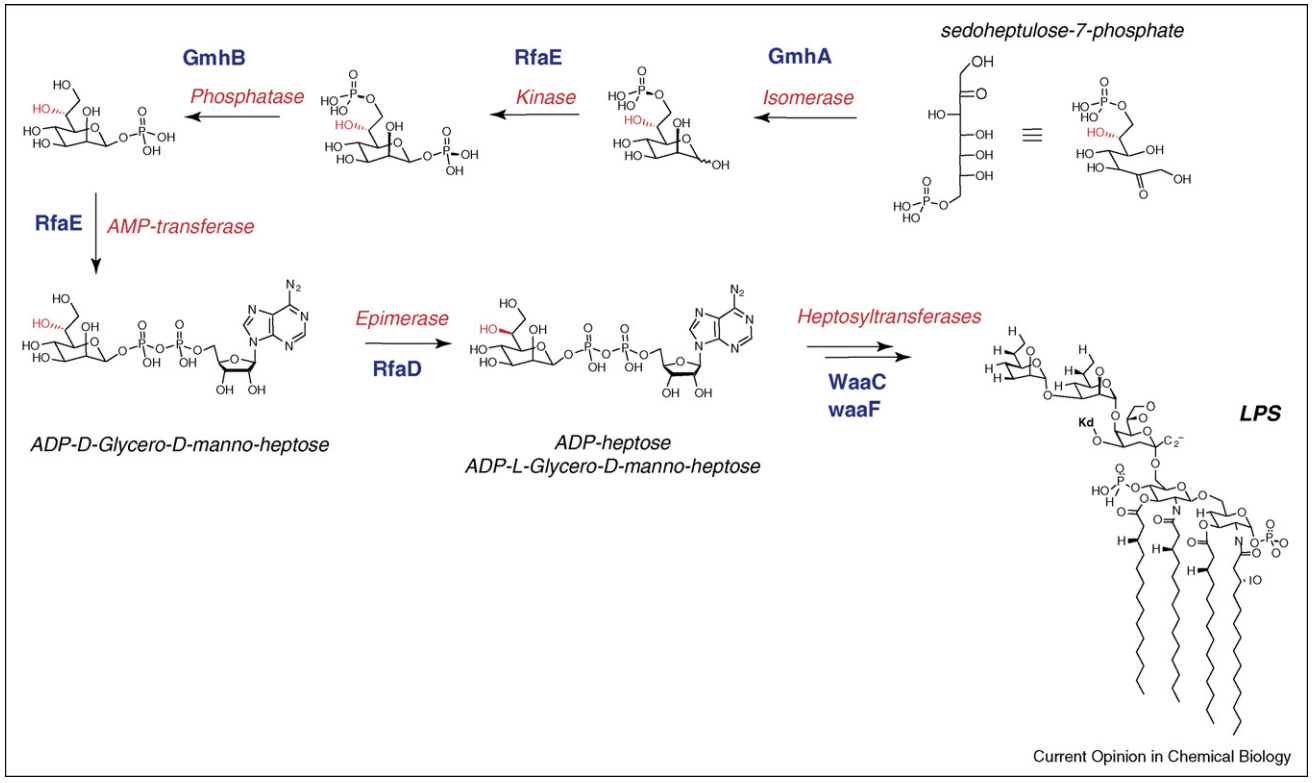
Among the many virulence factors described to be important for bacteria to resist, the innate immunity components are the mechanisms involved in resistance to cationic antimicrobial peptides (CAMP) [62]. The CAMP dominating targets are bacterial membranes, and CAMP interacts with the negatively charged bacterial surface. In Gram-positive pathogens, the antibacterial activities of the cationic peptides decrease as the bacterial electropositive charge surface increases [63,64].

Survival of bacteria to various bactericidal compounds including CAMP, cell killing, and cell invasion ability has been correlated to the D-alanylation of the lipotechoic acids (LTA) [65–67]. D-alanylation of LTA is mediated by the dlt operon (Figure 2) that is conserved in Gram-positive species of medical importance such as the staphylococci and streptococci.

The role of the dlt operon for sensitivity to cationic peptides and virulence has led to considering dlt genes, in particular DltA, as good targets in the search to find inhibitors [67].

A substrate derivative of DltA (D-alanylacyl-sulfamoyl-adenosine) was found to be a potent inhibitor of the enzyme. When used *in vitro* the inhibitor was active in rendering different *Bacillus subtilis* strains more sensitive to vancomycin [68].

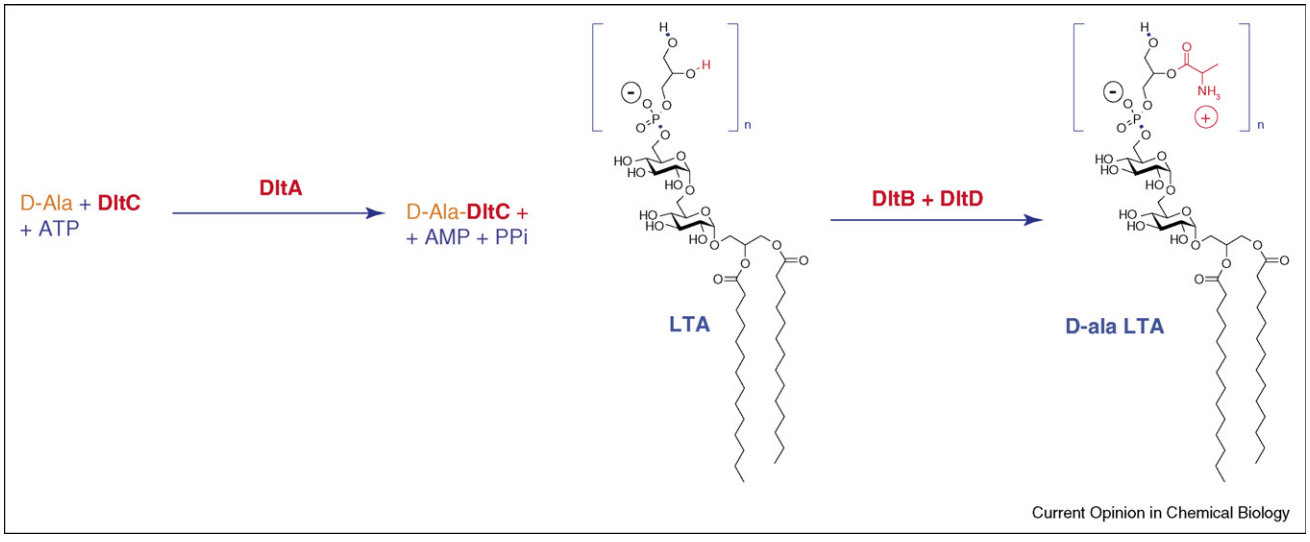
Figure 1



Heptose and inner core LPS biosynthesis in Gram-negative bacteria.

In Mutabilis, a series of potent inhibitors of *S. agalactiae* and *S. pyogenes* DltA was identified [69]. *In vitro*, the imidazolo-quinoxaline compound has no antibacterial activity by itself but was able to sensitize the bacteria to the lytic effect of cationic peptides. The peptide used in this *in vitro* assay was colistin that mimics the effect of antibacterial cationic peptide. The bacterial growth inhibition observed *in vitro* in presence of colistin was

Figure 2



Scheme of D-alanylation of lipotechoic acids (LTA) in Gram-positive bacteria.

correlated to the inhibition of the target enzyme, as measured by the decreased amount of D-ala present in the LTA of the bacterial cell wall after incubation with the compound. In an experimental model of systemic infection of mice by *S. agalactiae*, the DltA inhibitors were able to affect the bacterial multiplication in the host, as shown by a dose dependent decrease of bacteremia. The *in vivo* antibacterial effects of the compounds were obtained at doses that are comparable to the effective doses of classic antibiotics [70]. These data represent a first proof of concept that an antivirulence molecule has an antibacterial effect *in vivo* by making the bacteria sensitive to host defenses.

Another proof of concept for an antivirulence molecule against *S. aureus* has been published recently [71••]. A specific virulence factor for *S. aureus* is staphyloxanthin a pigment with antioxidant activity necessary to protect the bacteria from the ROS produced by phagocytic defense of the host; a potent phosphosulfonate inhibitor of staphyloxanthin biosynthesis was shown to decrease bacteremia in a systemic model of infection in mice. These results demonstrate that it is possible to render the pathogenic *S. aureus* sensitive to innate immunity and obtain an antibacterial effect. However, the inhibition of the interaction with host immune defenses needs to be validated in the human host.

Conclusions

These given examples of new chemical entities active against virulence targets that are able to induce an *in vivo* antibacterial effect demonstrate that new drugs can potentially be found to fight bacterial infections (Table 1). However, there are several issues for developing antivirulence drugs.

One of the main issues is the spectrum of activity. So the development of such drugs relies on the use of rapid diagnostic methods. With the availability of a large number of sequenced bacterial genomes, it is now possible to select virulence targets that are conserved in several species. Examples of such antivirulence inhibitors active on more than one species demonstrate the feasibility of this approach.

Another issue for the use of antivirulence molecules is the timing of expression of the virulence determinants during the infectious process. An ideal target would have a constitutive expression rather than inducible.

A main issue for drug discovery is the need for *in vitro* pharmacological assays to detect and optimize chemical inhibitors. Since virulence inhibitors by definition will not kill bacteria *in vitro*, functional assays should mimic the *in vivo* conditions necessary for the expression of the virulence phenotype and should be amenable to standardization. This key point is addressed in the selection of the virulence targets such as the resistance to soluble immune effectors such as CAMP or complement.

Potential indications will depend on the virulence mechanism targeted and on the spectrum of activity of the

Table 1

Examples of small molecules inhibitors of virulence mechanisms described in the cited literature

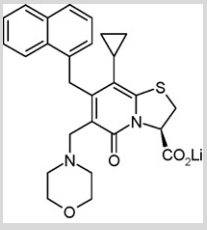
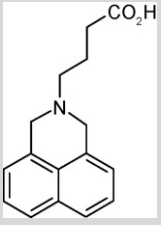
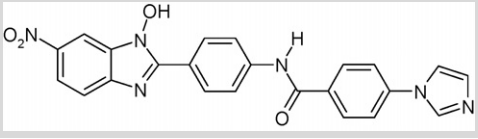
Reference number	Name	Structure
[20]	Compound 2C	
[21]	Virstatin	
[23]	P005301	

Table 1 (*Continued*)

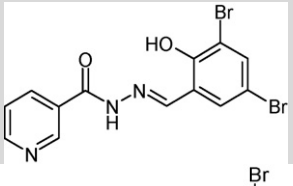
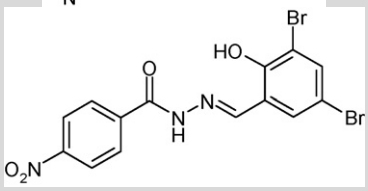
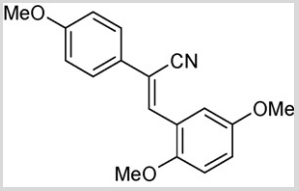
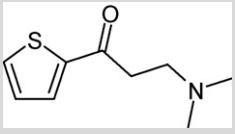
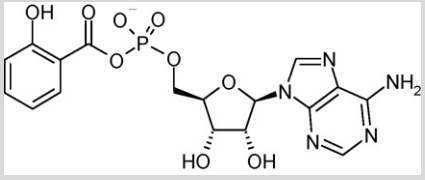
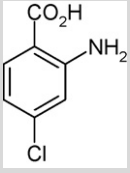
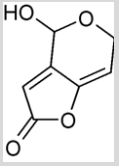
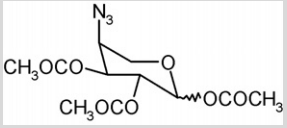
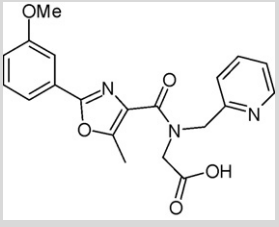
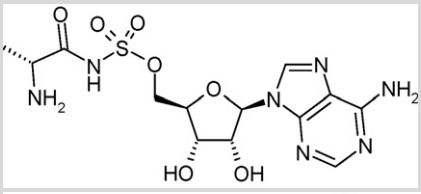
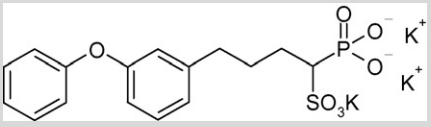
Reference number	Name	Structure
[24]	D9	
[27]	Compound 2	
[31]	Diarylacrylonitrile	
[33]	AAEK	
[34••]	AMS1	
[39]	4CABA	
[41]	Patulin	
[57•]	Compound 1	
[61]	MUT2585	

Table 1 (*Continued*)

Reference number	Name	Structure
[68]	Sulfamoyl D-ala	
[71••]	BPH652	

compounds. The choice would be based on the selective site of bacterial replication where the inhibition is needed (local or systemic).

For the molecules targeting the pili or the T3SS functions in enterobacteria, the clinical application would be to inhibit the bacterial multiplication at a specific site such as the intestine or the kidney.

For the antivirulence molecules addressing factors necessary for bacteria to avoid destruction by the immune system could be used to treat or prevent bacteremia.

With antivirulence compounds affecting the cell wall composition, the bacteria are also more sensitive to antibiotics, so combination therapy with current antibiotics could be envisioned.

References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Terzulli LS, Croft CA, D'Antoni VA: **Update on the antibacterial resistance crisis**. *Med Sci Monit* 2007, **13**:103-118.
2. Alanis JA: **Resistance to antibiotics: are we in the post-antibiotic era?** *Arch Med Res* 2005, **36**:697-705.
3. Croft CA, D'Antoni VA, Terzulli LS, Alanis JA: **Resistance to antibiotics: are we in the post-antibiotic era?** *Arch Med Res Med Sci Monit* 2005, **13(36)**:697-705 RA103–118.
4. Walsh F, Amyes S: **Microbiology and drug resistance mechanisms of fully resistant pathogens**. *Curr Opin Microbiol* 2004, **7**:439-444.
5. Payne D, Tomasz A: **The challenge of antibiotic resistant bacterial pathogens: the medical need the market and prospects for new antimicrobial agents**. *Curr Opin Microbiol* 2004, **7**:435-438.
6. • Payne JD, Gwynn NM, Holmes JDL, Pompliano D: **Drug for bad bugs: confronting the challenges of antibacterial discovery**. *Nat Rev* 2007, **6**:29-40.

The authors give an overview of the latest target-based approach used to identify new classes of antibiotics and discuss the HTS outcomes.

7. Talbot HG: **What is in the pipeline for Gram-negative pathogens?** *Expert Rev Anti Infect* 2008, **6**:39-49.
8. Alekshun NM, Levy SB: **Targeting virulence to prevent infection: to kill or not to kill?** *DDT* 2004, **1**:483-489.
9. •• Marra A: **Targeting virulence for antibacterial chemotherapy: identifying and characterising virulence factors for lead discovery**. *Drugs RD* 2006, **7**:1-16.

A good review of the main technology developed to investigate virulence targets.

10. Götz F: **Staphylococci in colonization and disease: prospective targets for drugs and vaccines**. *Curr Opin Microbiol* 2004, **7**:477-487.
11. Cegelski L, Marshall GR, Eldridge GR, Hultgren SJ: **The biology and future prospects of antivirulence therapies**. *Nat Rev Microbiol* 2008, **6**:17-27.
12. Escaich S: **A new therapeutic approach for the treatment of severe infections**. *Drug Plus Int* 2006, **5**:6-7.
13. Benton B *et al.*: **Large-Scale identification of genes required for full virulence of *Staphylococcus aureus***. *J Bacteriol* 2004, **186**:8478-8489.
14. Begun J *et al.*: ***Staphylococcus aureus* virulence factors identified by using a high-throughput *Caenorhabditis elegans*-killing model**. *Infect Immun* 2005, **73**:872-877.
15. Kurz CL, Ewbank JJ: **Infection in a dish: high-throughput analyses of bacterial pathogenesis**. *Curr Opin Microbiol* 2007, **10**:10-16.
16. Freiberg C, Brötz-Oesterhelt H: **Functional genomics in antibacterial drug discovery**. *Drug Discov Today* 2005, **10**:927-935.
17. Burrack SL, Higgins ED: **Genomic approaches to understanding bacterial virulence**. *Curr Opin Microbiol* 2007, **10**:4-9.
18. Sasakawa C, Hacker J: **Host–microbe interaction: bacteria**. *Curr Opin Microbiol* 2006, **6**:1-4.
19. Aberg V, Sellstedt M, Hedenström M, Pinkner JS, Hultgren SJ, Almqvist F: **Design, synthetics and evaluation of peptidomimetics based on substituted bicyclic 2-pyridones-targeting virulence of uropathogenic *E. coli***. *Bioorg Med Chem* 2006, **14**:7563-7581.
20. Pinkner SJ, Remaut H, Buelens F, Miller E, Aberg V, Pemberton N, Hedenström M, Larsson A, Seed P, Waksman G *et al.*: **Rationally designed small compounds inhibit pilus biogenesis in uropathogenic bacteria**. *Proc Natl Acad Sci* 2006, **103**:17897-17902.
21. Shakhnovich EA, Hung DT, Pierson E, Lee K, Mekalanos JJ: **Virstatin inhibits dimerization of the transcriptional activator ToxT**. *Proc Natl Acad Sci* 2007, **104**:2372-2377.
22. Galán JE, Wolf-Watz H: **Protein delivery into eukaryotic cells by type III secretion machines**. *Nature* 2006, **444**:567-573.
23. Draper MP, Garrity-Ryan LK, Donovan P, Kim OK, Grier M, Amoo V, Gay R, Hanley B, Bartell V, Macone A *et al.*: **A novel anti-virulence approach for treatment of pneumonia caused**

by *P. aeruginosa*. In *Proceedings of the 47th Interscience Conference on Antimicrobial Agents and Chemotherapy*; *Chicago, Illinois, USA, 17–20 September: 2007. (Abstract F2-968a)*.

24. Negrea A, Bjur E, Ygberg SE, Elofsson M, Wolf-Watz H, Rhen M: **Salicylidene acylhydrazides that affect type III protein secretion in *Salmonella enterica Serovar typhimurium***. *Antimicrob Agents Chemother* 2007, **8**:2867-2876.

25. Hudson DL, Layton AN, Field TR, Bowen AJ, Wolf-Watz H, Elofsson M, Stevens MP, Galyov EE: **Inhibition of Type III secretion in *Salmonella enterica Serovar typhimurium* by small-molecule inhibitors**. *Antimicrob Agents Chemother* 2007, **51**:2631-2635.

26. Nordfelth R, Kauppi AM, Norberg HA, Wolf-Watz H, Elofsson M: **Small-molecule inhibitors specifically targeting type III secretion**. *Infect Immun* 2005, **73**:3104-3114.

27. Wolf K, Betts J, Chellas-Géry B, Hower S, Linton CN, Fields KA: **Treatment of *Chlamydia trachomatis* with a small molecule inhibitor of the Yersinia type III secretion system disrupts progression of the chlamydial developmental cycle**. *Mol Microbiol* 2006, **61**:1543-1555.

28. Marraffini LA, DeDent AC, Schneewind O: **Sortases and the art of anchoring proteins to the envelopes of Gram-positive bacteria**. *Microbiol Mol Biol Rev* 2006, **1**:192-221.

29. Maresso WA, Schneewind O: **Sortase as a target of anti-infective therapy**. *Pharmacol Rev* 2008, **60**:128-141.

30. Kruger RG, Barkallah S, Frankel BA, McCafferty DG: **Inhibition of the *Staphylococcus aureus* sortase transpeptidase SrtA by phosphinic peptidomimetics**. *Bioorg Med Chem* 2004, **12**:3723-3729.

31. Ki-Bong O, Soo-Hwan K, Jaekwang L, Won-Jea C, Taeho L, Sanghee K: **Discovery of diarylacrylonitriles as a novel series of small molecule Sortase A inhibitors**. *J Med Chem* 2004, **47**:2418-2421.

32. Kang SS, Kim JG, Lee TH, Oh KB: **Flavonols inhibit sortases and sortase-mediated *Staphylococcus aureus* clumping to fibrinogen**. *Biol Pharm Bull* 2006, **29**:1751-1756.

33. Maresso AW, Wu R, Kern JW, Zhang R, Janik D, Missiakas M, Duban D, Joachimiak ME, Schneewind AO: **Activation of inhibitors by sortase triggers irreversible modification of the active site**. *J Biol Chem* 2007, **282**:23129-23139.

34. •• Ferreras JA, Ryu JS, Di Lello F, Tan SD, Quadri ENL: **Small-molecule inhibition of siderophore biosynthesis in *Mycobacterium tuberculosis* and *Yersinia pestis***. *Nat Chem Biol* 2005, **1**:29-32.
The authors present a very interesting rational design of inhibitor based on the common structural element of the substrate and the first active siderophore inhibitor.

35. Kong KF, Vuong C, Otto M: **Staphylococcus quorum sensing in biofilm formation and infection**. *IJMM* 2006, **296**:133-139.

36. Ventre I, Goodman LA, Vallet-Gely I, Vasseur P, Soscia C, Molin S, Bleves S, Lazdunski A, Lory S, Filloux A: **Multiple sensors control reciprocal expression of *Pseudomonas aeruginosa* regulatory RNA and virulence genes**. *Proc Natl Acad Sci* 2006, **103**:171-176.

37. Hentzer M, Wu H, Andersen BJ, Riedel K, Rasmussen BT, Bagge N, Kumar N, Schembri AM, Song Z, Kristoffersen P *et al.*: **Attenuation of *Pseudomonas aeruginosa* virulence by quorum sensing inhibitors**. *EMBO J* 2003, **22**:3803-3815.

38. Wu H, Song Z, Hentzer M, Andersen BJ, Molin S, Givskov M, Hoiby N: **Synthetic furanones inhibit quorum-sensing and enhance bacterial clearance in *Pseudomonas aeruginosa* lung infection in mice**. *J Antimicrob Chemother* 2004, **53**:1054-1061.

39. Lesic B, Lepine F, Deziel E: **Inhibitors of pathogen intercellular signals as selective anti-infective compounds**. *Plos Pathog* 2007, **3**:1229-1239.

40. Christensen DL, Moser C, Jensen P, Rasmussen BT, Christophersen L, Kjelleberg S, Kumar N, Hoiby N, Givskov M, Bjarnsholt T: **Impact of *Pseudomonas aeruginosa* quorum sensing on biofilm persistence in an *in vivo* intraperitoneal foreign-body infection model**. *Microbiology* 2007, **153**:2312-2320.

41. Rasmussen BT, Skindersoe EM, jarnsholt BT, Phipps KR, Christensen BK, Jensen OP, Andersen BJ, Koch B, Larsen OT, Hentzer M *et al.*: **Identity and effects of quorum-sensing inhibitors produced by Penicillium species**. *Microbiology* 2005, **151**:1325-1340.

42. Bronner S, Monteil H, Prévost G: **Regulation of virulence determinants in *Staphylococcus aureus*: complexity and applications**. *FEMS* 2004, **28**:183-200.

43. •• Otto M: **Quorum-sensing control in Staphylococci—a target for antimicrobial drug therapy?** *FEMS Microbiol* 2004, **241**:135-141.
the authors give a comprehensive overview of the knowledge of QS in *S. aureus* and the issues related to its inhibitions.

44. •• Camargo IL, Gilmore MS: ***Staphylococcus aureus*—probing for host weakness?** *J Bacteriol* 2008, **190**:2253-2256.
This review presents the latest understanding of the different sensing systems, including the nutrient sensitivity in *S. aureus* and how natural environments affect the behavior of bacteria that infect humans.

45. George AE, Novick PR, Muir WT: **Cyclic peptide inhibitors of staphylococcal virulence prepared by fmoc-based thiolactone peptide synthesis**. *J Am Chem Soc* 2008, **130**:4914-4924.

46. Balaban N, Cirioni O, Giacometti A, Ghiselli R, Braunstein BJ, Silvestri C, Mocchegiani F, Saba V, Scalise G: **Treatment of *Staphylococcus aureus* biofilm infection by the quorum-sensing inhibitor RIP**. *Antimicrob Agents Chemother* 2007, **51**:2226-2229.

47. •• Park J, Jagasia R, Kaufmann FG, Mathison CJ, Ruiz ID, Moss AJ, Meijler MM, Ulevitch JR, Janda DK: **Infection control by antibody disruption of bacterial quorum sensing signaling**. *Chem Biol* 2007, **14**:119-1127.
A very original study that demonstrates that antibodies directed to Agr could affect bacterial multiplication *in vivo*.

48. Sakoulas G, Eliopoulos MG, Fowler G, Moellering CR, Novick PR, Lucindo N, Yeaman RM, Bayer SA: **Reduced susceptibility of *Staphylococcus aureus* to vancomycin and platelet microbicidal protein correlates with defective autolysis and loss of accessory gene regulator (agr) function**. *Antimicrob Agents Chemother* 2005, **49**:2687-2692.

49. Tsuji TB, Rybak JM, Lau LK, Sakoulas G: **Evaluation of accessory gene regulator (agr) group and function in the proclivity towards vancomycin intermediate resistance in *Staphylococcus aureus***. *Antimicrob Agents Chemother* 2007, **57**:1089-1091.

50. Yarwood MJ, Paquette MK, Tikh I, Volper ME, Greenberg EP: **Generation of virulence factor variants in *Staphylococcus aureus* biofilms**. *J Bacteriol* 2007, **189**:7961-7967.

51. Ryback JM: **Pharmacodynamics relation to antimicrobial resistance**. *AJIC* 2006, **34**:38-45.

52. Porat Y, Marynka K, Tam A, Steinberg D, Mor A: **Acyl-substituted Dermaseptin S4 derivatives with improved bactericidal properties, including on oral microflora**. *Antimicrob Agents Chemother* 2006, **50**:4153-4160.

53. • Brown KL, Hancock RE: **Cationic host defense peptides**. *Curr Opin Immunol* 2006, **18**:24-30.
This review presents the different mechanism of actions that are associated to the antimicrobial activity of host peptides.

54. Mdluli KE, Witte PR, Kline T, Barb AW, Erwin AL, Mansfield BE, McClerren AL, Pirrung MC, Tumey LN, Warrener P *et al.*: **Molecular validation of LpxC as an antibacterial drug target in *Pseudomonas aeruginosa***. *Antimicrob Agents Chemother* 2006, **50**:2178-2184.

55. Adachi H, Kondo K, Kojima F, Umezawa Y, Ishino K, Hotta K, Nishimura Y: **Synthesis and inhibitory activity of 8-substituted 2-deoxy-beta-KDO against CMP-KDO synthetase**. *Nat Prod Res* 2006, **20**:361-370.

56. Rosenfeld Y, Shai Y: **Lipopolysaccharide (Endotoxin)-host defense antibacterial peptides interactions: role in bacterial resistance and prevention of sepsis**. *Biochim Biophys Acta* 2006, **1757**:1513-1522.

57. Kline T, Trent MS, Stead CM, Lee MS, Sousa MC, Felise HB, • Nguyen HV, Miller SI: **Synthesis of and evaluation of lipid A modification by 4-substituted 4-deoxy arabinose analogs as potential inhibitors of bacterial polymyxin resistance**. *Bioorg Med Chem* 2008, **18**:1507-1510.
This paper presents an interesting strategy of molecule design to inhibit LPS modification and induce polymyxin resistance.

58. Harper M, Cox AD, St Michael F, Wilkie IW, Boyce JD, Adler B: **A heptosyltransferase mutant of Pasteurella multocida produces a truncated lipopolysaccharide structure and is attenuated in virulence**. *Infect Immun* 2004, **72**:3436-3443.

59. Kim CH: **A *Salmonella typhimurium* rfaE mutant recovers invasiveness for human epithelial cells when complemented by wild type rfaE (controlling biosynthesis of ADP-L-glycero-β-D-manno-heptose-containing lipopolysaccharide)**. *Mol Cells* 2003, **15**:226-232.

60. De Leon GP, Elowe NH, Koteva KP, Valvano MA, Wright GD: **An *in vitro* screen of bacterial lipopolysaccharide biosynthetic enzymes identifies an inhibitor of ADP-heptose biosynthesis**. *Chem Biol* 2006, **13**:437-441.

61. Escaich S, Moreau F, Le Fralliec G, Denis A, Gerusz V, Desroy N, Floquet S: **Discovery of new Gram-negative antivirulence drugs. 2 Characterization of novel *E. coli* RfaE inhibitors**. In *Proceedings of the 46th Interscience Conference on Antimicrobial Agents and Chemotherapy*; *San Francisco, CA, USA, 27–30 September: 2006. (Abstract F2-496)*.

62. Boman HG: **Antibacterial peptides: basic facts and emerging concepts**. *J Intern Med* 2003, **254**:197-215.

63. Poyart C, Pellegrini E, Marceau M, Baptista M, Jaubert F, Lamy CM, Trieu-Cuot P: **Attenuated virulence of *Streptococcus agalactiae* deficient in D-alanyl-lipoteichoic acid is due to an increased susceptibility to defensins and phagocytic**. *Cells Mol Microbiol* 2003, **49**:1615-1625.

64. Peschel L, Otto M, Jack WR, Kalbacher H, Jung G, Götz F: **Inactivation of *dlt* operon in *Staphylococcus aureus* confers sensitivity to defensins, protegrins, and other antimicrobial peptides**. *J Biol Chem* 1999, **274**:8405-8410.

65. Kovacs M, Halfmann A, Fedtke I, Heintz M, Peschel A, Vollmer W, Hakenbeck R, Brückner R: **A functional dlt operon, encoding proteins required for incorporation of D-alanine in teichoic acids in Gram-positive bacteria, confers resistance to cationic antimicrobial peptides in *Streptococcus pneumoniae***. *J Bacteriol* 2006, **188**:5797-5805.

66. Fabretti F, Theilacker C, Baldassarri L, Kaczynski Z, Kropec A, Holst O, Huebner J: **Alanine esters of enterococcal lipoteichoic acid play a role in biofilm formation and resistance to antimicrobial peptides**. *Infect Immun* 2006, **74**:4164-4171.

67. Weidenmaier C, Peschel A: **Teichoic acids and related cell-wall glycopolymers in Gram-Positive physiology and host interactions**. *Nat Rev Microbiol* 2008, **6**:276-287.

68. May JJ, Finking R, Wiegeshoff F, Weber TT, Bandur N, Koert U, Marahiel MA: **Inhibition of the D-alanine:D-alanyl carrier protein ligase from Bacillus subtilis increases the bacterium's susceptibility to antibiotics that target the cell wall**. *FEBS J* 2005, **272**:2993-3003.

69. Denis A, Moreau F, Vongsouthi V, Prouvensier L, Genevard JM, Durant L, Bonvin Y, Gerusz V, Escaich S: **Discovery of new Gram-positive antivirulence drugs1: *in vitro* characterization of novel DltA inhibitors**. In *Proceedings of the 47th Interscience Conference on Antimicrobial Agents and Chemotherapy*; *Chicago, Illinois, USA, 17–20 September: 2007. (Abstract F2-957)*.

70. Escaich S, Moreau F, Malacain E, Vongsouthi V, Soulama C, Saccomani M, Prouvensier L, Oxoby M, Gerusz V, Denis A: **Discovery of new Gram-positive antivirulence drugs2: the first antivirulence molecule active *in vivo***. In *Proceedings of the 47th Interscience Conference on Antimicrobial Agents and Chemotherapy*; *Chicago, Illinois, USA, 17–20 September: 2007. (Abstract F2-958)*.

71. Liu CI, Liu YG, Song Y, Yin F, Hensler EM, Jeng WY, Nizet V, •• Wang HJA, Oldfield E: **A cholesterol biosynthesis inhibitor blocks Staphylococcus areus virulence**. *Sci Exp* 2008, **319**:1391-1394.
The paper presents important results demonstrating the validity of anti-virulence as a new therapy against *S. aureus*.